THIRD LAYER OF AMOEBA VS. TRICHOCYSTS OF PARAMECIUM.*

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INTRODUCTION.

We are often asked by students and co-workers why amoebæ with no weapons of defense can capture and digest *Paramecium caudatum* and other protozoa when these organisms possess such weapons as trichocysts (Fig. 3).

We believe we can answer the above question from observations we have made. In these observations, which will be described in detail in the following pages, we have found that, when an Amoeba has succeeded in killing a Paramecium, the former organism was mature in practically every case. An immature amoeba was unsuccessful according to our observations, since the third layer was not thick enough to protect it from the trichocysts of the Paramecium. We feel the third layer is the essential structure used by the Amoeba in securing food and, therefore, we have described the characteristics of that layer in the following paragraphs.

THE THIRD LAYER.

That a third layer exists on an Amoeba was indicated by observations made by Butschli (2) in 1892 and by Blochmann (1) in 1894. Several years later Gruber (4) recognized the same structure and described it as a permanently differentiated layer composed of gelatinous substance. Schaeffer (12) in 1917 called the third layer one of protoplasm which moves faster than the forward advance of the Amoeba and later gave a very clear description of its movements as indicated by its ability to carry particles.

Jennings, (5) on the other hand, concluded from his observations that no third layer existed, but that particles clinging to the outside of the Amoeba were carried toward the anterior end by the ectoplasm.

*We are indebted to Doctor H. O. Halvorson for many helpful criticisms in preparing this paper.

We are convinced, however, that a third layer does exist and that the age of the Amoeba determines the thickness of this layer. We are further convinced that the thickness of the third layer is an important factor in determining the kind of food secured by the Amoeba. In this connection, we noticed in our cultures that a very young Amoeba lives upon bacteria,

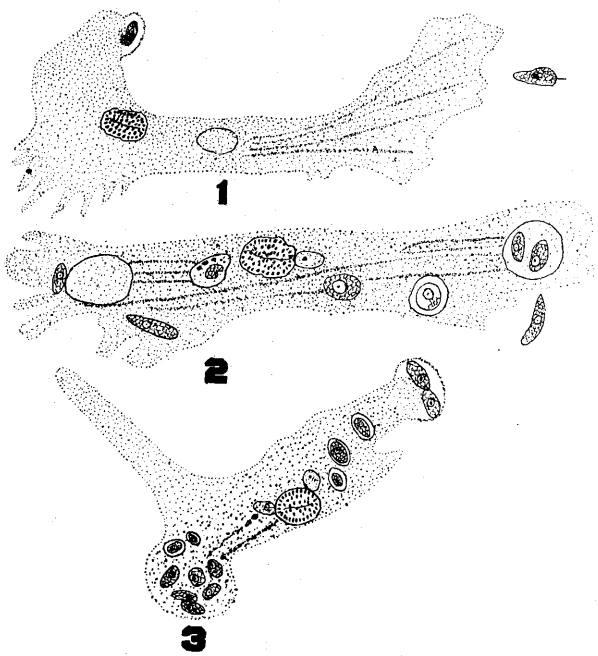

FIG. 1. Amoeba with its third layer surrounding a Chilomonas paramecium.
FIG. 2. Chilomonas paramecia with the third layer of an amoeba surrounding them forming food vacuoles.
FIG. 3. The third layer of an amoeba surrounding two Chilomonas paramecia at the extreme right. The third layer disappears as the food vacuoles approach the nucleus. At the posterior end of the amoeba there are numerous Chilomonas paramecia. These were caught when the amoeba was flowing in the direction opposite to the one shown in the diagram.

when older it chooses *Chilmonas paramecium* (Text Figs. 1, 2, 3), later paramecia is the principal diet, and finally Rotifers are selected.

According to our observations practically all conflicts between mature Amoeba and Paramecium were fought to the advantage of the former organism. On the other hand a young Amoeba, in such a battle, always lost. While working on the life cycle of *Amoeba proteus*, (Jones (6), we observed in

one of Dr. Kepner's cultures a conflict between a young Amoeba, a Paramecium and another protozoan, *Urotricha farcata*. In this skirmish, the Amoeba attacking the Paramecium, began to project pseudopodia almost immediately on either side of the Paramecium but before they had extended far, it put out another in the opposite direction as if to move away. In the meantime, *U. farcata* came up to the Paramecium and stopped, then it tried to move away but its efforts were feeble. The Paramecium by means of its trichocysts killed both the Amoeba and *U. farcata*. At this point the Paramecium was killed with osmic acid fumes and fixed as described below.* In the photograph (Fig. 1), the reader can see the trichocysts discharged into the Amoeba and *Urotricha* and that the latter is a little swollen in the direction of the Paramecium. It is of interest to note the Paramecium apparently discharged only those trichocysts which came in contact with the Amoeba and *Urotricha*.

Although, as previously stated, we feel that the age of the Amoeba and, therefore, the amount of third layer present is extremely important in determining the kind and size of food secured by the organisms, we have also noticed that even a mature Amoeba is not always successful in killing a Paramecium. Sometimes the latter is able to free itself without difficulty from an Amoeba. Such an escape, we are confident is due to the absence of a necessary amount of third layer on the surface of the Amoeba at the time the Paramecium is caught. When the Amoeba has caught several organisms, each of those animals takes with it to the interior of the Amoeba a certain amount of third layer, (Text Figs. 2, 3). As a result, if several organisms have previously been caught and, therefore, several food cups formed in the Amoeba, there naturally will be less of the third layer on the surface. Under such a condition the Amoeba is of course less able to hold its prey, and often the latter gets away. Kepner and Whitlock (10) show a good example of this in Plate III (Fig. 12). In this illustration it is evident that an Amoeba caught a euglena after several other organisms has previously been secured. The euglena escaped, a condition due, undoubtedly, to an insufficient amount of third layer on the surface of the Amoeba at the time the flagellate was caught.

*The organisms were killed with osmic acid fumes, fixed in Schaudinn solution (Saturated $HgCl_2$ in 95 per cent alcohol plus acetic acid) and stained in Heidenhain's iron-alum-haematoxylin (short method).

The small amount of third layer present on the surface was needed by the Amoeba to protect the ectoplasm and endoplasm of its body from the lashing flagellum of the euglena.

There are no indications of the third layer possessing any power of digestion, since no signs of erosion occur until all the third layer has left the food vacuoles or has been digested by the enzymes in the endoplasm.

After protozoa have been caught and taken into the Amoeba, the food vacuole containing the imprisoned organisms passes towards the nucleus. During this passage the third layer, taken in the food vacuole with the organisms, gradually disappears and is entirely gone by the time the vacuole reaches the nucleus (Text Figs. 3). The contents of the food vacuole are then digested and the indigestible particles pass away from the nucleus and out of the Amoeba at the posterior end.

When an Amoeba catches protozoa at one end and then reverses its direction of flow, such as illustrated in Text figure 3, the organisms caught remain at the posterior end until the movements of the Amoeba cease or are reversed again.

TRICHOCYSTS.

Paramecium in defending themselves against Amoeba, project short stout rods called trichocysts. Although there is little definite information about these rods it is known that they lie within the ectoplasm. A few investigators are of the opinion that trichocysts are used chiefly for protection, others feel they must be weapons used for offense while still others hesitate to credit them with either function. Mast (11) supporting the theory that the Paramecium uses its trichocysts for protection, discusses the behavior of the organism when encountering its worst enemy, the *Didinium*. The latter attacks its prey by means of a peristome which can be discharged as an elongated tubler proboscis. If it succeeds in fixing this structure to a Paramecium it is usually able to suck much of its prisoner's body into its own. According to Mast (11), when the Paramecium is attacked by a *Didinium*, it discharges its trichocysts, which form a great tangled mass about the body of its enemy. This discharge of trichocysts, in the cases of the largest Paramecia forms such a dense mass that the *Didinium* is pushed free from the Paramecium and the latter thus escapes death.

Our observations coincide with those of Mast, but we have also noticed that the trichocysts are entirely liberated from the body of the Paramecium when it is attacked by a *Didinium* but are not liberated when it is attacked by other animals. Such a condition, we feel, must be due to a stimulus caused by an injection of material from the proboscis of the *Didinium*. Figures 1, 3, shows trichocysts discharged but still attached to the Paramecium.

A MATURE AMOEBA ENGULFS A PARAMECIUM WITH ITS THIRD LAYER.

When a Paramecium is not moving about but has its cilia in motion, currents are set up by the cilia which become swifter as they pass from the anterior to the posterior end of the animal. If an Amoeba is near enough the Paramecium to receive a stimulus from these currents, the first pseudopodium formed by the Amoeba will be pointed toward the posterior end of the Paramecium, since this is the source of greatest stimulus. As the Amoeba moves nearer the Paramecium, other pseudopodia of smaller size are formed. These are right and left of the first pseudopodium. When a pseudopodium touches the Paramecium the third layer of that pseudopodium immediately engulfs the Paramecium.

In catching a Paramecium or any other animal of similar structure, it is necessary for the third layer of the Amoeba to first surround or engulf the emprisoned organism. Naturally according to this method of securing food, it is necessary for the Amoeba to have plenty of third layer.

Kepner and Whitlock's (10) drawings, reproduced on Plate III (Figs. 9, 10, 11), describe the catching of a Paramecium by an Amoeba as follows: The amoeba was advancing in a general way toward the Paramecium along pseudopodia 1, 2 and 3. As it approached the ciliate, pseudopodia 1 and 2 widened and partly fused to form a large bi-lobed extremity, m-ml. When this extremity had nearly touched the Paramecium, it sent out a small psudopodium, beneath the prey, and b anterior to it (Fig. 10). When the pseudopodia, a and b came in contact with the detritus, y, they moved apart and become much stouter (Fig. 11). In the meantime a third pseudopodium, e, appeared projecting from between a and b over the dorsal side of the Paramecium, while a pocket was formed within the body

proper of the amoeba at the bases of these three pseudopodia. The Paramecium first jumped to position 2, Fig. 11. The excited Paramecium next backed into the pocket of the body proper, 3 and a, b, and e, closed in and surrounded it completely."

Our interpretations of the diagrams described above differ from those given by Kepner and Whitlock, since we bring into use the third layer. According to our interpretations the Paramecium was still, but its cilia were moving violently, judging by the amount of debris that was passing along the side and back of the posterior end of the Paramecium. The stimulus thus set up caused the Amoeba to put forth pseudopodium number 2 first, 3 next, then one, since this was the order of the stimulus as it was received by the Amoeba; notice, number 1 would touch the Paramecium about the center; 2 is off at the posterior end, while 3 would be useless in catching the Paramecium. The next in order, number 4, would be out of consideration. As 1 and 2 approached nearer the Paramecium, the third layer of either pseudopodia 1 and 2 or both would flow around and engulf the Paramecium. Figure 4, text figures 1 and 2, shows the third layer of an Amoeba surrounding *Chilomonas paramecium* in this same fashion.

The engulfing of a paramecium by the third layer may be due to the adhesive forces. The third layer having a strong affinity for the surface of a paramecium tends to flow over and around it. This same flow may cause a shifting of surface tension forces, which will account in part, at least, for the withdrawal of extended pseudopodia as in Plate III, Fig. 10. Part of the third layer having flowed around the Paramecium will tend to level out, due to surface tension forces, thus drawing the Paramecium into the cytoplasm of the Amoeba or, visa versa, the cytoplasm of Amoeba around the Paramecium. As a result, secondary pseudopodia flow up through the third layer and around the paramecium finally engulfing the organism along with some of the third layer as pictured by Kepner and Whitlock (10), Plate III, Fig. 11. These authors, in observing this condition, however, pictures the Amoeba surrounded by a layer of water instead of protoplasmic substance. According to our observations, third layer and not water surrounded the engulfed organism as we were able to stain the material, (Fig. 4, Text figures 1, 2, 3).

Kepner and Whitlock (10) state "that an Amoeba may react to a quiet paramecium in three ways; (1) by forming a pocket within its own body within which the ciliate will be driven (Figs. 9, 10, 11, 12), by sending encircling pseudopodia about the prey and then roofing over and flooring the enclosed space with ectoplasm before disturbing the prey, and (3) by closing in upon the Paramecium with the advancing tips of two pseudopodia until the prey is held fast in a grip of the pseudopodia ends. After the Paramecium is thus caught, it is very tightly closed in upon and constricted."

We have observed that the pseudopodia will only extend around the third layer. In other words, the pseudopodia will not encircle a living protozoan until the third layer has completely surrounded the prey.

We have also seen mature Amoeba catch *Chilomonas paramecium* in the manner described by Kepner and Whitlock (10) but in every instance observed in stained preparations, the third layer had surrounded the *Chilomonas paramecium* before the cup of the Amoeba began to close.

SUMMARY.

1. Paramecium can kill young amoeba with its trichocysts.

2. Mature amoeba has a thicker third layer than an immature one which protects the ectoplasm and endoplasm from the trichocysts of other protozoa.

3. Trichocysts are not thrown off from a paramecium when attacking amoeba and Urotrichia as described by Mast when *Didinium* attacks the paramecium.

4. Pseudopodia will not extend around an organism until the third layer has first surrounded it.

5. The organism caught by an amoeba is carried towards the nucleus, the third layer in the food vacuole disappears as digestion takes place.

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EXPLANATION OF PLATES.

PLATE I.

Fig. 1. Paramecium with its trichocysts discharged, killing a young amoeba and a Urotricha.

Fig. 2. *Amoeba proteus* showing the third layer surrounding the amoeba. The amoeba is filled with food vacuoles. Notice the two Chilomonas paramecia. The one at the posterior end shows signs of erosion, while the one at the large end shows no such change. The dark bodies, except the large one which is the nucleus, are food vacuoles in different degrees of digestion.

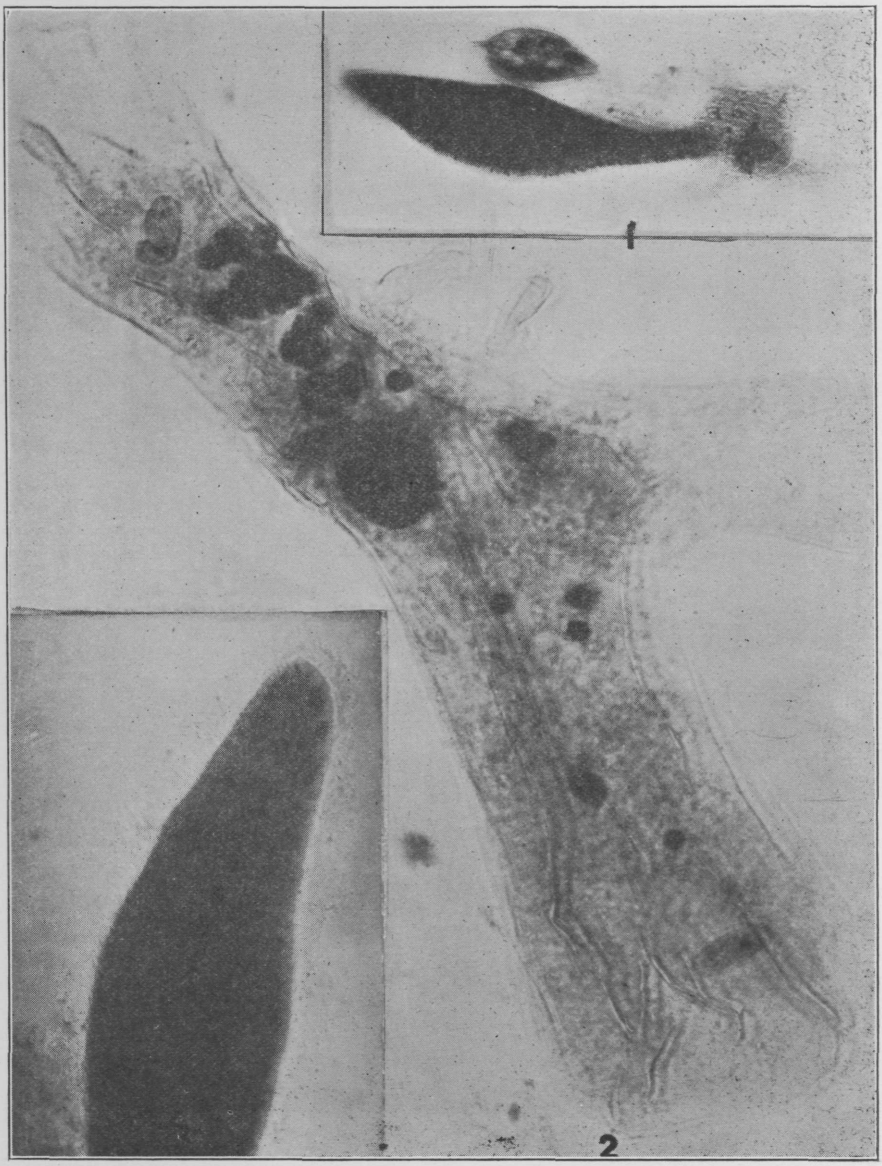
Fig. 3. Paramecium cordatum with its trichocysts extended; the capsules are stained black with iron heamatoxylin.

PLATE II.

Fig. 4. Amoeba catching active moving Chilomonas paramecia by surrounding them with the third layer on the arms while it is catching a chilomonas in a food cup at the larger end.

PLATE III.

Figs. 9, 10 and 11. Shows an amoeba catching a paramecium as explained by Kepner and Whitlock. (Reproduced by permission of Dr. Kepner.)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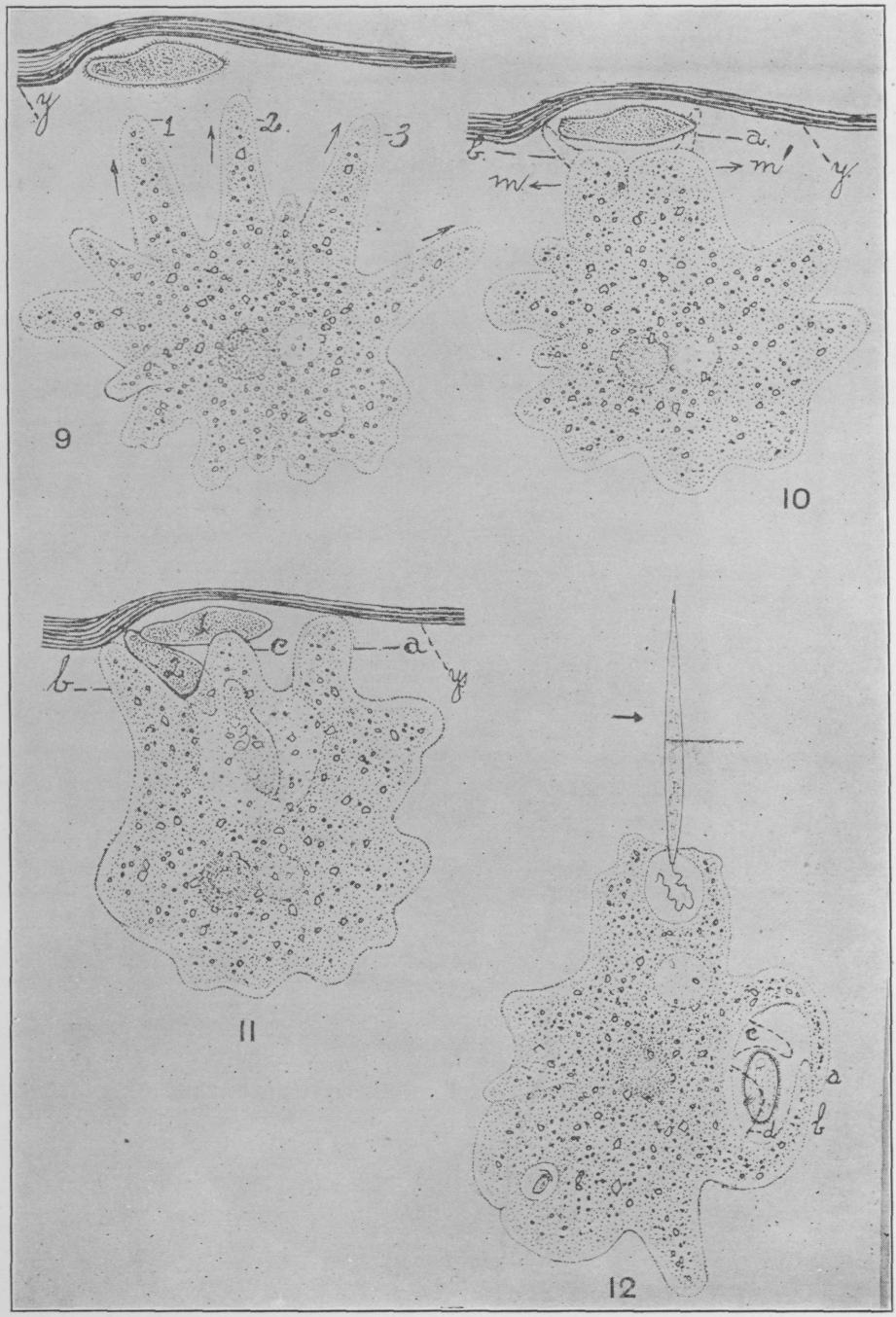
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